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THE THRESHOLD CENTRIFUGE

By

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This dissertation is a description of the principle and construction of the threshold centrifuge and a discussion and summary of the following papers:

1. **RASTGELDI, S., A new continuous centrifuge for isolation and differentiation of particles in a suspension, Scandinav. J. Clin. & Lab. Invest, vol. 10, suppl. 31, 301, 1957.**

2. **RASTGELDI, S., Isolering och differentiering av formelement i pleuraexsudat, ascites m. m. med hjälp av en ny anordning för centrifugering, Svenska läk.tidn. 54, 2655, 1957.**

3. **RASTGELDI, S., Differentiering av erytrocyter med hjälp av kammarcentrifug, Svenska läk.tidn. 54, 2655, 1957.**

4. **RASTGELDI, S. and TURANLI, I., Concentration of malignant cells from vaginal washings by means of a new type of centrifuge, Acta obst. et gynec. scandinav. 37, 392, 1958.**

5. **RASTGELDI, S., The simultaneous separation and concentration of corpuscular elements and bacteria from human urine by means of a new type of centrifuge, Acta chir. scandinav. 116, —, 1958.**

6. **RASTGELDI, S., TOMENIUS, J. and WILLIAMS, G., The threshold centrifuge as a means for concentrating cells aspirated by gastric washings. Acta med. scandinav. 160, —, 1958.**

Preface

The present work has been carried out at the Chemistry Department II of the Karolinska Institutet in collaboration with the Central Laboratory of the Sabbatsbergs Sjukhus, the Cytological Department of the Radiumhemmet, the Urological Department of the Karolinska Sjukhuset and the Outpatient Department of S:t Görans Sjukhus, Stockholm.

The centrifuge models presented in this investigation have been constructed in collaboration with the centrifuge firm of Winkelcentrifug, Stockholm.

To my chief, Professor Erik Jorpes, I am deeply indebted for his kind interest in my work and the help he has given me in every direction during this investigation.

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Introduction

The application of centrifugal force for practical purposes was not unknown in ancient and medieval times—e.g. for the separation of honey from beeswax and the separation of tung oil in a similar way.

In 1669, the Dutch physicist Christian Huygens submitted to the Royal Society of London the results of investigations he had made concerning centrifugal force (de vi centrifuga). A report of these investigations was published four years later, in 1673.

In 1820, Jonathan Shoffield constructed the first centrifuge for drying textile fabrics, consisting of a rapidly rotating drum around which the wet fabric was wrapped. The centrifuge proper, however, came into being in 1836, when a German piano builder in Paris, Gottfried Penzoldt, constructed an apparatus »for drying woolen and other fabrics.» His first machine consisted of a perforated cylindrical drum made of tin, and was called by him »machine à rotation.» The wet fabric was placed in the centrifugal drum and upon rotation of the drum the water was pressed out through the holes.

Penzoldt made some very important improvements of the centrifuge. He collaborated with his employer Johann Gottlob Seyrig, a German mechanic who owned the piano workshop in Paris, and together with him constructed a centrifuge which was driven by a steam turbine attached to the shaft of the rotating drum.

The main problem in which Penzoldt became deeply engaged was the elimination of vibration in the rapidly rotating drums. For this purpose he constructed various devices for running the centrifuges smoothly, free from vibrations which could result in their explosion.

A few years after the construction of the perforated centrifuge drums for drying fabrics, imperforate drums were constructed and used mainly in the sugar industry. Lawrence Hardman described, in 1843, »Certains perfectionnements dans les machines ou appareils employés dans la fabrication du sucre.»

Paraf and Bazile wrote, in 1849, »Application de la force centrifuge à la precipitation des corps en suspension dans les liquides» and for the first time showed that the particles in a suspension could be precipitated quickly by application of centrifugal force.

Albert Fesca, a well-known Berlin machine manufacturer, con-

structed, in 1852, a centrifuge in which he used Penzoldt's ring regulators and hammers, which were later known as Fesca's ring regulators. Fesca's centrifuges were used for many years in Europe, especially in the production of sugar.

It was, by this time, well known that cream is nothing but small particles of fat dispersed in the heavier components of milk. C. J. Fuchs in Karlsruhe was, as far as it is known, the first to propose separation of cream from milk by applying centrifugal force. He constructed, in 1859 a small centrifuge for the quantitative determination of cream in milk. In 1864, a brewer in Munich, A. Prandtl, constructed a centrifuge with obliquely mounted tubes for the separation of cream from milk. A similar centrifuge was constructed in 1872 by Moser. These two centrifuges show a very interesting construction. Both of them are of a multitubular pattern and of angular design. Although the cream could be separated from milk by running the centrifuge at a very low speed, 400—600 r.p.m., the advantage of using angular tubes was not discovered and a multitubular system was not found useful in the separation of cream from milk. These prototypes were later modified by W. Lefeld who constructed, in 1874, a centrifuge which consisted of a disk on the periphery of which a number of tubes could be mounted.

As early as 1874, a continuous centrifuge, the »decantation centrifuge continue,» was constructed at »La Compagnie de Fives Lille» in France, and described to work as follows: »Ils seront caracterisés par la marche continue des appareils, par la sortie simultanée et en marche des diversees matières, separés les unes des autres.» These continuous centrifuges were intended to be used in the separation of wine. Since clarification of wine was the dominating problem in France, no one thought of separating cream from milk, although this could have been done. However, these continuous centrifuges never came into use in the wine industry, because the first prototype exploded and killed the inventor!

Meanwhile, Lefeld carried on his centrifuge experiments mainly by modifying the imperforate rotating drums, which were used extensively in the sugar industry. In 1876, he constructed a new centrifuge which could separate cream from milk. This centrifuge, however, did not operate continuously.

It was, finally, the Swedish engineer Gustaf de Laval who, in 1878, succeeded in constructing a continuous centrifuge which separated cream from milk very efficiently. This centrifuge was first called »the cream separator,» then »the centrifugal separator,» and finally only »the separator.»

In 1890, the separator was improved by the addition of von Bechtolsheim's conical plates, known as the Alfa plates.

Although, in 1849, Paraf and Bazile had shown that the particles in a suspension could be precipitated quickly by means of centrifugal force, the centrifuge was not applied in medicine until, towards the end of the nineteenth century, when the Swedish physiologist Gustaf Magnus Blix constructed the first prototype of the haematocrit. In 1889, this centrifuge was modified and used by Sven Gustaf Hedin for the rapid and accurate determination of the relative volume of the red blood corpuscles.

Ragnar Lundgren, a Swedish »medicine kandidat,» improved the sedimentation effect in the centrifuge tubes by positioning the tubes obliquely in relation to the shaft of the centrifuge. He designed, in 1927, the first prototype of the angle centrifuge which has since then been used on a very large scale in clinics and laboratories. He deduced the angle principle from his observations on the rate of sedimentation of blood, which was considerably more rapid in inclined tubes than in vertical tubes. He proposed to measure the sedimentation rate of the erythrocytes in inclined tubes, but this idea never gained popularity. The value of the angle centrifuge, however, as developed by the Swedish technologist Gunnar Beckman (who has constructed angle centrifuges since 1927) was soon appreciated.

The angle principle has been applied by Danielsson & Lundmark in their spiral centrifuge, which consists of a long spiral groove in the disk-shaped rotor of the centrifuge in which is placed a long thin plastic tube containing blood. Danielsson & Lundmark have reported a satisfactory concentration of leukocytes by means of the spiral centrifuge, which is at the same time used as a haematocrit centrifuge.

In the beginning of the 1920's, Svedberg constructed the ultracentrifuge for the determination of the molecular weights of proteins and other macromolecules.

In 1948, Lindahl described the principle of the counter-streaming centrifuge for the separation of particles and cells of different size.

As far as can be ascertained by reviewing the literature in this field, the threshold principle has not been utilized in the various centrifuge types mentioned above. The principle of separating particles of different size and weight by treating them in a stream of air or running water has been known for many thousands of years, e.g. in washing cereals in running water or in panning gold

in order to separate small pieces of the precious metal from the less valuable and lighter sand and earth.

The present author began to experiment with centrifuges towards the end of 1954. It was planned to construct a centrifuge for the purpose of finding out if it was possible to separate macromolecular proteins and polysaccharides from each other by letting them glide on very rapidly rotating surfaces in a continuous centrifuge. The first prototype constructed for this purpose was destroyed by explosion at the end of 1954. Neither the experience nor the resources of the author permitted continuation of the experiments at the time. It was then decided to construct low speed centrifuges for the purpose of separating different cells and bacteria from each other by the same principle, viz. by letting the particles glide on rotating surfaces. It was soon found that a smooth surface was not sufficient for separating different particles from each other. The rotating surfaces were then provided with a large number of ridges or thresholds. From the first experiment with such a threshold centrifuge it was apparent that particles of different size and weight could be efficiently separated from each other by using »thresholds.»

The principle of the threshold centrifuge is so simple, and the apparatus is in practice so easily handled that the author felt that it could be used to advantage in pathological diagnosis where an examination of cells and bacteria from different body fluids is a prerequisite. During 1956—1958 the author has been mainly engaged in the construction of low speed threshold centrifuges. Experiments with high speed centrifuges were laid aside, for the time being. Now two prototypes of high speed centrifuges driven by compressed air and running at a rate of more than 40,000 r.p.m. have been constructed and some tentative experiments for separating particles of macromolecular size have been made.

The threshold centrifuges can be constructed by modifying a centrifuge of the rotating-cylinder type or a centrifugal separator. The author has, however, chosen the simple centrifuge tube, which is the most familiar type of centrifugal device to the physician and the chemist, as an object of comparison in the explanation of the principle and construction of the threshold centrifuge.

PART I

The principle and construction of the threshold centrifuge

Ordinary centrifuge tubes are inaccessible during centrifugation, i.e. it is not possible to fill the centrifuge while it is in operation. An ordinary centrifuge tube is restricted to a certain volume and its purpose is to keep the particles sedimented by the centrifugal force, in one layer of the tube, and the suspending agent in another. The possibilities of separating different particles according to their size and weight are limited. *Figure 1* shows 10 centrifuge tubes before centrifugation. Each contains a suspension of two different kinds of particles evenly dispersed. After centrifugation the particles are separated from the suspending agent as shown in *Figure 2*. The lighter particles float on top of the heavier ones and the original contents of the tubes are divided into three different layers, the suspending agent on top, the lighter particles in the middle and the heavier particles at the bottom of the centrifuge tubes. Theoretically these three sections can be separated from each other by suction. The lighter particles can then be conveyed to an extra tube and isolated from the bulk of the larger particles remaining in the tubes 1—10 *(Fig. 3)*.

When the series of the centrifuge tubes shown in *Figs. 1—3* are connected to each other as shown in *Figure 4* and, while the centrifuge is in operation, filled progressively from the beginning of the series *(Fig. 5)*, the heavier particles will be retained in the tubes at the beginning of the series and the lighter particles floating on top of the heavier ones will be conveyed from one tube to the other as the tubes are progressively filled. Under ideal conditions the lighter particles will be quantitatively or nearly quantitatively separated from the heavier ones *(Fig. 6)*.

In the course of the centrifugation, first the heavier particles and then the lighter ones sink to the bottom of the centrifuge tubes. If the suspended particles constitute a high percentage of the total volume, particles of different sizes will usually be packed together. As a consequence, the heavier particles cannot freely penetrate towards the bottom of the tubes and for the same reason the lighter ones cannot rise to the uppermost layer. This is almost what happens when blood is centrifuged at a high r.p.m.

This inconveniency can be partly eliminated by constructing shallow centrifuge tubes of large cross-sectional area, as shown in *Fig. 7*. In this modification the depth of the centrifuge tubes is diminished so that the heavier particles can penetrate towards the bottom and lift the lighter ones. *Figure 8* shows a long channel which is divided into a large number of compartments or chambers corresponding to a large number of intercommunicating centrifuge tubes which are to be filled progressively from the beginning of the channel.

Figure 9 shows a series of compartments arranged circularly in the rotor of a centrifuge. The suspension is introduced from the center of rotation and the compartments are filled progressively during operation.

Figure 10 shows a segmented channel obtained by laying a long plastic tube against the inner wall of a rotating drum provided with a large number of vertical ridges. During centrifugation the ridges press the corresponding points on the peripheral wall of the plastic channel towards the centre and divide the tube into a large number of compartments which correspond to a large number of intercommunicating centrifuge tubes. The central part of the channel is formed as a half circle so that the particles can glide on the smooth plastic surface without getting damaged.

Figure 11 shows the deposit of particles in the compartments during centrifugation, and *Figure 12* shows the same deposit after the centrifuge has stopped rotating and the plastic tube has regained its original shape.

The plastic tube can be cut into segments corresponding to one or more compartments, *Figure 13*. The sediment of particles can be exposed by cutting the plastic tube longitudinally *(Figure 14)*. The heavier particles will be found at the beginning of the tubing and the lighter ones towards the end.

Figures 1—14 give a short description of the principles underlying this centrifuge. A more concise description as to the construction of the different models will now be given.

Introduction of a suspension into the centrifuge:

In *Figure 15*, the tubular shaft of the centrifuge is designated by 1. A tubing 3, of a flexible material, for instance rubber, forming a circle or a helix in the centrifuge is introduced through a slot 2 and drawn out through the motor shaft the upper portion of which is threaded, so that the lid of the rotor can be screwed on to it. When the centrifuge reaches a certain speed, a suspension or a solution is run directly into the hose through its free

end. When leaving the motor shaft 1 the hose 3 forms an angle to the motor shaft. Due to this angle the entering suspension or suspending agent is thrown from the central end of the tubing towards its peripheral end. The suspension may be introduced through a tube, by a syringe or the like.

In *Figure 16*, the hollow motor shaft 1 is widened to form an elliptical chamber 2a. This chamber communicating with the tubing 3 is filled by means of a narrow tube. The suspension is thrown against the peripheral wall of the chamber and from there into the tubing.

Figure 17 shows an annular chamber 2b concentrical to the motor shaft 1. The upper part of the chamber 2b is open, and a narrow tube can be introduced into the annular opening during centrifugation. The tubing 3 is filled in the same way as stated above.

Figure 18 shows a bent tube 2c separated from the centrifuge shaft 1, the upper portion of this tube being passed through the lid 2d of the centrifuge. The lower portion of the tube communicates with the central end of the tubing 3. The tubing is filled through the tube 2c during centrifugation.

Some designs of the multitubular rotors and threshold centrifuges:

Figure 19 shows a centrifuge with a length of tubing 3 wound helically around a truncated cone 4. A conical casing 5 is applied outside the cone 4 so that the tubing 3 is retained between the two concentric surfaces.

Figure 20 is a section of *Figure 19* in which a number of longitudinal ridges 6 are provided in the outer casing 5. During centrifugation the tubing 3 is pressed against the casing 5. Those portions of the tubing which come into contact with the ridges 6 are constricted, and between two constrictions pockets or chambers 7 are formed which communicate with each other. When a suspension is introduced during centrifugation the heavier particles are pressed against the peripheral wall of the tubing and the lighter ones float above the heavier ones. The suspending agent, flowing in at a constant velocity, conveys the lighter particles from one chamber to the other. The suspension having been centrifuged, the outer casing is removed and the hose is then accessible.

Figure 21 shows a circularly wound tubing which forms right angles to the motor shaft. The centrifugal force increases progressively in each coil from the shaft 1 towards the periphery. The

heavier particles remain in the central part and the lighter ones in the peripheral part of the tubing 3, when the coils are provided with ridges on the peripheral side of each coil.

Figure 22 shows a ring 8 concentric to the motor shaft. Around this ring a hose 3 is wound which is then passed through the shaft 1 of the centrifuge. In this model, it is possible to use, inter alia, two liquids insoluble in each other.

Figure 23 shows a number of pillars or bolts 11 which are fixed on a rotor. The tubing 3 can be wound progressively around each pillar or be passed between the pillars in such a manner that the hose forms a number of loops. This model is designed to be used for counter current chromatography as well as for separating different solid elements from each other.

Figure 24 illustrates a model in which a spiral extends from the shaft 1 of the centrifuge. The tubing 3 is placed in a spirally formed groove 9. A number inwardly facing ridges 6 are formed in the peripheral wall of the groove, a great number of intercommunicating chambers 7 thereby being formed in the tubing. The peripheral end of the tubing is left open. The suspending agent is thrown out through the peripheral end of the tubing and the heavier solid particles will stick to the peripheral wall of the tubing 3.

Figure 25 shows a diagram of the groove made in the rotor illustrated by Figure 24. The chambers 7a are provided in the peripheral wall of the groove. During centrifugation the suspending agent will be conveyed in the direction indicated by the large arrows and the suspended particles in the direction indicated by the small arrows. In this model the tubing may be dispensed with.

Figure 26 shows another model of a groove 9a made in the disk.

Figure 27 shows a rotor comprising an inner cylinder 12 and an outer rotating casing 13, the inner wall of which is provided with a number of ridges 6. A length of plastic tubing 3 is wound around the inner cylinder 12 which is then placed in the ridged casing 13. The plastic tube is drawn through the tubular centrifuge shaft 1 and cut above the shaft. The suspension is fed at a constant rate during centrifugation and the chambers or compartments provided in the tubing are filled progressively from the upper end of the inner cylinder towards the lower end.

Figure 28 is a cross section of *Figure 27* along the line XIV—XIV.

Figure 29 is a photograph of the centrifuge, the details of which are described in *Figs. 27* and *28*.

Figure 30 is a modification of the rotor shown in *Figs. 27* and

28. The tubing 3 is laid in the circular groove 16 and then slipped over the end of a bent metal or plastic tube 17 communicating with the central chamber 2a shown in *Figure 31.* The peripheral wall of the circular groove is provided with a number of ridges 6 which divide the tubing 3 into a corresponding number of intercommunicating chambers 7. The suspension is introduced through a tube 18 in the central chamber 2a communicating with the tubing 3.

Figure 31 is a cross section of *Fig. 30* along the line XVI—XVI.

The centrifuge shown in *Fig. 32* comprises a cylindrical container 19 rigidly connected to the shaft 1 of the motor. A cylinder 20 provided with concentric, inwardly directed ridges 21 is inserted into the container 19. The ridges 21 correspond to the abutments 6 of the models mentioned above. The container 19 is provided with a lid 22 and suspension or liquid is supplied to the uppermost chamber 7 through the lid 22 by means of the hose 3. The inlet opening in the lid is journalled in a ball-bearing 23. The direction of the flow of the solid particles or the precipitate is indicated by the fine arrows and the heavy arrows indicate the direction of the flow of the liquid. The insert 20 is preferably formed of plastic material which can be cut into different sections after the centrifugation.

The model shown in *Figs. 33* and *34* comprises a tube 24 secured coaxially to the shaft of the motor. A number of sockets 25 are connected to this tube 24 and the centrifuge tubes 26 are threaded on them. The centrifuge tubes 26 correspond to the chambers 7. The suspension is supplied by means of a hose 18.

Figure 35 is a modification of *Figure 24.*

Figure 36 shows the annular passage 2b provided around the perforated shaft of the centrifuge and communicating with the spiral tubing 3.

Figure 37 shows a length of the tubing 3 placed in a circular or spiral groove 9. The section 7 of the tubing 3 on each side of a ridge or threshold 6 correspond to intercommunicating shallow centrifuge tubes.

Figure 38 shows a cross section through the disk shown in *Fig. 35.* The suspension is introduced through a tube 18 into the annular passage 2b provided around the tubular perforated shaft 1 of the centrifuge and communicating with the tubing 3. During centrifugation, the suspension is thrown against the inner wall of the annular passage 2b and from there into the tubing 3. The compartments shown in *Fig. 37* are filled progressively from the beginning of the rotating channel and towards the end.

Figure 39 is a perspective view of the centrifuge, the details of which have already been described in *Figs. 24, 25* and *35—38.*

Figure 40 is a perspective view of a centrifuge with a rotating drum 27 which has been perforated by a large number of holes 28 on the upper edge and also a corresponding number of holes on the lower edge. A variable number of ridges 6 is obtained by passing a thread or a plastic coated wire consecutively through the holes. The plastic tube 3 is laid in the rotating drum in a number of coils and finally pulled over a metal or plastic tube 29 connecting the tubing 3 with a removable metal or plastic tube 30 placed in the tubular and threaded centrifuge shaft 1. The inner cylinders or cones shown in *Figs. 19* and *27* can be dispensed with in this model. Intercommunicating chambers 7 are formed during centrifugation and the circularly laid tubing 3 is held in its place both during and after centrifugation. Parts 29—30 can be removed by drawing them upwards so that the thinner and bent metal or plastic tube 29 can glide through the slot 2 shown both in this figure and in *Fig. 15.*

Figure 41 shows a centrifuge rotor which comprises numerous horizontal bolts 31 corresponding to bolts shown in *Fig. 23* and a number of oblique holes 32. This model is a compound centrifuge and has been used both as a multitubular threshold centrifuge and as a common angle centrifuge. The hose 3 is drawn over a thin metal or plastic tube 29a which is continuous with a shorter removable part 29, 30. The tubing 3 is then passed between the upper and lower row of the bolts 31 so that a number of »U»-bends is obtained. One or several plastic rings 6a corresponding to the ridges shown in the previous figures are placed around the rotor, thus compressing the »U»-bends of the tubing 3. Two or more intercomunicating compartments 7 are obtained between one upper and one lower bolt 31.

Figure 42 shows the same centrifuge *(Fig. 41)* with the outer casing 13a snugly fitting around the bolts 31. This model has been constructed for the purpose of eliminating turbulence in the rotating channel 3 during the critical moment of vibration before the centrifuge stops.

Figure 43 is a photograph of the same centrifuge as shown in *Figs. 41* and *42.* Here the particles are to be seen in the lower parts of the curves and the suspending agent in the upper parts. A section of the rotating channel 3 between two upper bolts is cut and placed in the oblique holes 32 *(Fig. 41).* The contents of this section corresponding to four or more intercommunicating chambers are centrifuged conventionally and the concentrate of

particles is separated from the suspending agent by cutting the sections at the border between the sediment and the suspending agent.

Figure 44 is a perspective view of the centrifuges described in *Figs. 40—43* in operation, with the addition of a drop counter 33 and the lid 34. The suspending agent stripped of solid particles runs through the opening 35 and is collected in a separate container. A plexiglass window 36 is provided at the top of the centrifuge lid 34 to enable one to see that the inlet tube 18 operates satisfactorily and conveys the suspension into the central opening of the rotating channel.

PART II

Previously published work

Some applications of the threshold centrifuge to pathological diagnosis

Paper 1

A new continuous centrifuge for isolation and differentiation of particles in a suspension.

In paper 1 a general description of the principle of the threshold centrifuge is given. The centrifuge models described in part one (in reference to *Figures 1—44*) are constructed on the basis of this principle.

Some of the centrifuge models shown by drawings and descriptions have not been used in this dissertation. All of the constructions fall, however, within the limits of the principle of the threshold centrifuge. This principle relates to a method of treating liquids or dispersions in a centrifuge in order to separate the components according to their density or solubility, by introducing the liquid or dispersion, during the operation of the centrifuge, into a channel provided in the rotor of the centrifuge and causing the liquid or dispersion progressively to pass a number of projections or THRESHOLDS provided in the path of the channel.

The centrifuge models based on this principle have been previously called the »Kammarcentrifug» (paper 3) in view of the chambers, Swedish »kammare», which are formed between the thresholds. The centrifuge has later been renamed »the threshold centrifuge», and will be referred to by this name in the future.

One model of the threshold centrifuge, in which the channel is formed by laying a plastic tubing in a spiral groove has been confused and mistakenly identified with the spiral centrifuge recently constructed by Danielsson and Lundmark (Nordisk Medicin, 1957, 57, 175, Nr. 5). An abstract from the original text in Nordisk Medicin is given below:

»The Spiral Centrifuge
A new type of centrifuge
by Harry Danielsson and Folke Lundmark.

A new type of centrifuge — called the spiral centrifuge — is described. Its principle component is a circular plexiglass disk, 15 cm in diameter, having a spiral groove in which is placed a transparent plastic tube 50 cm in length. The tube is filled with e.g. whole blood or a solution of blood and citrate. *The spiral serves in the same way as a straight centrifuge tube 50 cm long;*[1]

[1] **The present authors italics.**

inside it, particles are disposed in layers according to their specific gravities. Leukocytes, for instance, are visibly localized to a certain section of the tube. This is then severed at appropriate sites and the column of leukocytes can run down on to an object glass for microscopic analysis.»

Paper 2

Isolation and differentiation of cells and bacteria in pleural and ascitic exudates.

Paper 2 is an account of the isolation and separation of cells in pleural and ascitic exudates. The centrifuge models used in this investigation are those shown in *Figures 24—25* and *35—39*. A rotor provided with a spiral groove has been chosen to illustrate the functioning of the thresholds in separating heavier cells from lighter ones. In this model, the heavier cells sediment near the centre of rotation and the lighter ones are carried further away and deposited more peripherally. This deposition of heavier particles near the centre of rotation and the lighter ones near the periphery of the rotor is in complete contrast to any other centrifuge in which the heavier particles are found most remote from the centre of rotation and the lighter ones, which float on top of the heavy particles, near the centre of rotation. The tubing laid in a spiral groove in the threshold centrifuge is not used as an ordinary centrifuge tube for the following obvious two reasons:

1. the peripheral end of the tubing is left open whereas the peripheral ends of all common centrifuge tubes are closed.

2. the particles are not deposited throughout the tubing first at the far end and then towards the centre of rotation but only on the peripheral wall of the tubing first on the central part of the tubing and only later towards the peripheral end.

Other prototypes of the threshold centrifuge can be used for isolation and separation of the cellular elements in pleural and ascitic exudates. Centrifuge models shown in *Figures 27—31* and *40—44* have been found as useful as the spiral models in the examination of the cells in ascitic and pleural exudates. *Figure 45* is a photograph, showing how ascites can be tapped from a patient, and led directly to a centrifuge, where the cells can be simultaneously concentrated and separated. The centrifuge

shown is the model of *Figures 27—29*. Pleural exudates can be led to an operating centrifuge in the same way.

Intercellular adhesiveness must be considered when using any model of the threshold centrifuge. Sometimes it is very difficult or even impossible to make a slide from such clustered cells removed from the centrifuge. In such cases, however, some other method than the ordinary smear technique can be used.

Cell conglomerates or clusters, occassionally originating from a tumour, or pieces of fibrin, disturb fractionation of the cells. Such clusters or pieces can be prevented from entering the centrifuge by inserting a filter in the supply tube. They can later be removed from the filter and examined separately, preferably by a histological technique.

Pleural and ascitic exudates must be centrifuged without loss of time in order to prevent bacterial growth and cell deterioration. Even very small numbers of bacteria can be demonstrated by means of the threshold centrifuge, which makes it necessary to exclude aerial contamination as the source of the bacteria. The patient-to-centrifuge technique *(Fig. 45)* is recommended in all cases where bacterial infection is suspected. The bacteria can be concentrated from very large volumes and this technique can be very time saving in bacteriology.

Some adequate anticoagulant must be added to the freshly tapped pleural or ascitic exudates and the suspension must be lightly stirred during centrifugation in order to prevent the cells from sedimenting in the container. Haemorrhagic exudates can be diluted by physiological saline or Ringer's solution in order to facilitate the centrifugation.

Paper 3

Differentiation of erythrocytes.

In paper 3, a method for the separation of normal human erythrocytes according to corpuscular size and haemoglobin content is described. The threshold centrifuge models used in this investigation are those shown in *Figures 27—31*. Rotors shown in *Figures 19—20* which are described in the same paper have been found unsatisfactory, due to the fact that the tubing wound around the cone and kept under a well fitting conical casing slides down to the lower end of the casing during centrifugation.

In contrast to the cells in ascitic and pleural exudates, urine and vaginal washings, the erythrocytes do not stick to the peripheral wall of the tubing. Turbulence during the critical moment of vibration when the centrifuge stops or during removal of the tubing from the rotor is apt to cause mixing of the erythrocytes separated in different chambers during centrifugation. In order to prevent this turbulence and running of erythrocytes from one chamber to the other, the centrifuge models shown in *Figures 40—44* have been designed and constructed and have proved satisfactory.

In separating normal erythrocytes by corpuscular size and haemoglobin content, 10—15 ml blood is withdrawn from a vein into a centrifuge tube containing some suitable anticoagulant e.g. heparin. The blood is centrifuged conventionally and plasma and leukocytes are separated from the erythrocytes by suction. The plasma can later on be added to the suspension of erythrocytes but the leukocytes must be discarded. The erythrocytes are then stirred into 250—500 ml physiological saline, an even suspension being made. The suspension is then led to the already rotating threshold centrifuge.

After carefully removing the tubing from the centrifuge rotor, it is cut into equal pieces corresponding to one or several chambers. These pieces are then bent into a V-shape and placed in appropriate centrifuge tubes or in the oblique holes, as shown in *Figures 41* and *43*, and centrifuged once more. After this conventional centrifugation the compact erythrocyte sediment is separated from the suspending agent, physiological saline or a mixture of plasma and physiological saline, by cutting the V:s at the border between the suspending agent and the erythrocytes. It is assumed that the erythrocytes in different V:s are equally packed, i.e. give the same haematocrit value.

The erythrocytes are then transferred to small tubes and homogenized. The tubes are stoppered by rubber plugs in order to prevent evaporation. The number of erythrocytes and the haemoglobin content per unit volume, mm^3, are then determined.

It has been shown that the haemoglobin content per unit volume is highest at the beginning and lowest at the end of the tubing *(Fig. 46 a)*.

The number of erythrocytes per mm^3 increases progressively towards the end of the tubing, and at the same time the absolute amount of haemoglobin per erythrocyte decreases progressively *(Fig. 46 b—d)*.

Separation of erythrocytes according to corpuscular size and

haemoglobin content has been found valuable for the following reasons:

1. By separating erythrocytes according to corpuscular size and haemoglobin content it can be easily demonstrated that the threshold centrifuge actually separates particles with minute differences in density.

2. Easily obtainable erythrocytes can be used as standard corpuscles in investigating or experimenting with other cells.

3. By examining the erythrocytes before and after centrifugation one can draw the conclusion that these cells are not destroyed in the threshold centrifuge.

4. No haemolysis occurs in the threshold centrifuge and no haemoglobin is detected in the suspending agent, saline or a mixture of saline and plasma, ejected from the peripheral or distal end of the tubing. It is very remarkable that the erythrocytes can stand such a rapid acceleration after entering the central opening of a threshold centrifuge running at a speed of 6,000—8,000 r.p.m.

5. Contrary to other corpuscular elements, especially leukocytes, intercellular adhesiveness in normal erythrocytes is minimal or nonexistent. This is the main reason why the erythrocytes can be satisfactorily separated according to corpuscular size and haemoglobin content. In threshold centrifugation of erythrocytes the leukocytes must be excluded prior to centrifugation as described above. The leukocytes stick to each other and form small conglomerates which may prevent a satisfactory separation of the erythrocytes, and give unreliable haemoglobin values in the peripheral or distal chambers.

6. By determining the haemoglobin content of two equal amounts of erythrocytes before and after centrifugation the concentrating capacity of the threshold centrifuge can be easily demonstrated. After centrifugation of 0.1 ml erythrocytes suspended in 1,000 ml physiological saline about 70 % haemoglobin was recovered. In a corresponding experiment with 0.2 ml erythrocytes in 1,000 ml saline more than 90 % haemoglobin was recovered.

Paper 4

Concentration of malignant cells from vaginal washings.

In paper 4 a brief description is given of the concentration of malignant cells obtained from vaginal washings in cases of untreated carcinoma of the cervix and uterus. This work has been done in collaboration with Dr. I. Turanli.

A short survey of various methods for obtaining cells from the cervical canal and the endometrium is given, and it is pointed out that only a fraction of the cellular material present in the vagina is aspirated by the methods referred to.

Exfoliated cells have been collected by washing the vagina with warm physiological saline. A glass catheter of the type used in cystoscopy is connected to an irrigator containing physiological saline at body temperature. The catheter is introduced into the vagina and pushed against the posterior fornix. A flexible plastic funnel is held between the vulva and the anus and pressed against the perineum. A 500-ml bottle is held under the funnel. The stopcock of the catheter is opened and the vagina is irrigated by moving the catheter about the vagina. The patient lies in the dorsal position and the cell suspension from the vagina runs from the vulva through the funnel into the bottle. The vagina is irrigated until the bottle is filled *(Fig. 47)*.

The suspension of the exfoliated cells has been centrifuged by the models shown in *Figures 24, 25, 27—31* and *35—39*.

It is a well-known fact that cell remnants, detritus, bacteria and especially mucus make vaginal smears unsatisfactory. These disturbing elements have been satisfactorily separated from the intact cells by means of the threshold centrifuge.

In the absence of erythrocytes, undestroyed malignant cells sediment in the compartments at the beginning of the channel. A slide from the beginning of the channel will usually give a high concentration of malignant cells which have been cleaned of mucus, bacteria and detritus. In cases of haemorrhage, however, three or more slides from different parts, red as well as white sections, should be prepared. Pyknotic cells, free nuclei, cell remnans, bacteria, mucus and detriuts are found more distally or peripherally in the channel. In centrifuging very small amounts of cells, the number of thresholds in the beginning of the channel must be increased so that all solid elements do not fall into a single chamber without fractionation.

Cells exfoliated in groups or clusters of typical diagnostic importance were not in our experiments shaken apart and separated from each other by the threshold centrifuge.

Centrifuged slides from thirty patients have been compared with routine smears and it has been proved that the malignant cells do not lose their cytological identity during fractionation of the solid elements obtained by vaginal washings. Diagnostically important cells have been satisfactorily separated from erythrocytes, mucus, detritus and bacteria and concentrated together with some other cells having the same specific gravity and/or size. Microscopic slides prepared by the technique described have facilitated identification of the malignant cells.

Paper 5

The simultaneous separation and concentration of corpuscular elements and bacteria from human urine.

In paper 5 a method for the simultaneous separation and concentration of corpuscular elements and bacteria from human urine is described.

Centrifuge models used in this investigation are those shown in *Figures 24, 25, 27—31,* and *40—44.*

After placing a length of empty, clean plastic tubing in the rotor the centrifuge is started and all or part or a urine sample is run through the tubing. The ideal way of collecting and separating cells and bacteria from human urine is to let the patient urinate directly into a funnel connected to the already rotating threshold centrifuge *(Figure 48).* In this investigation urine samples have been collected in sterile bottles and transported to the laboratory without loss of time. Some bacteria may have grown in the urine before centrifugation but this point lies outside the scope of this investigation. After having centrifuged the urine sample the tubing is removed from the rotor and cut into pieces corresponding to one or more chambers or compartments. These pieces are then cut longitudinally in the proximal segments, the sediment is exposed and mixed with a small amount of 50 % fresh egg white and 50 % glycerin, a slide is made, fixed and stained by Papanicolaou's method. In the distal segments where free bacteria are found, Gram and Ziehl-Nielsen staining has been performed.

In the absence of crystalline and amorphous salts intact cells sediment at the beginning of the tubing. A slide from the beginning of the rotating channel will usually give a high concentration of cells which have been cleaned of mucus, bacteria and detritus. Pyknotic cells, free nuclei, cell remnants, bacteria, detritus and mucus are found more distally in the tubing. In the centrifugation of small amounts of urine the number of the thresholds in the beginning of the channel must be increased so that all solid elements do not fall into a single chamber without fractionation.

Free bacteria appear distally together with detritus and some cell remnants which form a thin semitransparent film on the peripheral wall of the tubing. Already deteriorated cells, free nuclei and cell remnants appear in the intermediate compartments between free bacteria and intact cells. Bacteria which have penetrated the cytoplasm of the cells cannot be separated from the cells and appear at the beginning of the tubing.

Particles in the chambers intermediate between intact cells at the beginning of the tubing and free bacteria towards the end contain a mixed population and have less diagnostic value both cytologically and bacteriologically.

The material investigated in this paper comprises urine samples from fifty different patients treated at the urological department of the Karolinska Hospital, Stockholm. In some cases, more than one sample was obtained from a patient. It is advantageous to centrifuge freshly voided urine without loss of time in order to prevent bacterial growth and cell deterioration occuring during preservation of the samples. It is preferable to centrifuge single portions of urine from the same patient instead of collecting larger portions during the day. The amount of urine has varied from 50 to 600 ml. Turbid cloudy urine samples have been diluted by adding an equal volume of physiological saline. Fresh urine of high specific gravity should be diluted with saline solution in order to preserve the cells and facilitate the sedimentation of bacteria at the distal end of the tubing. All patients had firm diagnoses prior to the centrifugation of the urine samples. It has been found that both cytologically and to a certain extent bacteriologically the microscopical findings are in agreement with the diagnosis.

Paper 6

The threshold centrifuge as a means for concentrating cells aspirated by gastric washings.

Paper 6 is an account of the concentration of cells aspirated from the stomach. This investigation has been carried out in collaboration with Docent John H. Tomenius and Dr. Gösta Williams at the Sankt Görans Hospital, Stockholm.

Nowhere else in the body are cells exposed to such strong deteriorating agents as in the stomach. The proteolytic enzymes, hydrochloric acid, osmotic changes etc. in the stomach commonly destroy the cells originating from the stomach as well as cells shed from the upper alimentary and respiratory tracts.

If the cytological identity of the cells found in the stomach is to be preserved, one must immediately transfer the cells into a milieu which has no further deteriorating effect. A simple and efficient way of preserving the cells is to dilute the contents of the stomach with a large volume of physiological saline or Ringer's solution both before and after aspiration. Further, aspirated and diluted stomach contents must be immediately centrifuged before any extensive deterioration takes place.

The following technique has been used in the isolation and concentration of cells from the stomach: The patient is not allowed to take any solid food for eight hours prior to aspiration of the stomach contents, whereas water and clear fruit juices are permitted.

A threshold centrifuge is put into operation, cool physiological saline from a large container (about 1 litre) is supplied to the central opening of the centrifuge.

The patient swallows a wide gastric tube with several large openings at the lower end. About 200 ml of saline or Ringer's solution from a large syringe are slowly injected through the gastric tube. Thereafter the contents of the stomach are aspirated carefully into the same syringe, from which they are then supplied to and mixed with the cool saline in the container connected to the already operating centrifuge. The same procedure of stomach lavage is repeated a number of times with subsequent immediate centrifugation, as before. Simultaneously, cool physiological saline is continuously fed to the container. If thick mucus is aspirated from the stomach it can be made more fluid by shaking it gently in an excess amount of cool saline or Ringer's

solution. After having centrifuged the whole amount, the container may be rinsed with an additional amount of cool saline or Ringer's solution.

The centrifuge is stopped, the contents are removed as previously described, and the slides are prepared, fixed, and stained by Papanicolaou's method.

Intact cells aspirated from the stomach are to be found at the beginning of the channel.

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Figures 1—48

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FIG.1

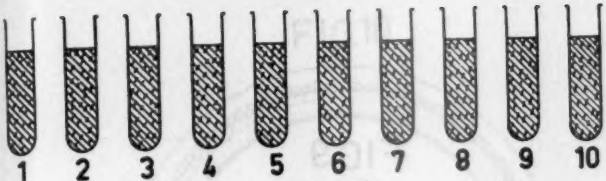
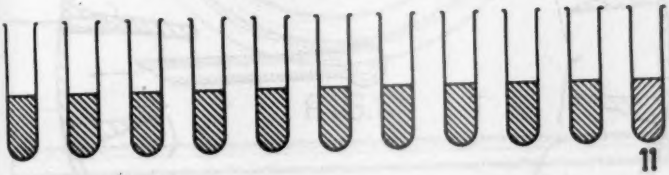


FIG.2

FIG.3



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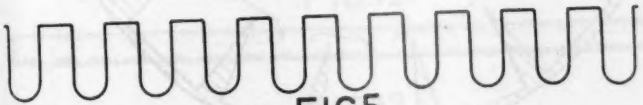

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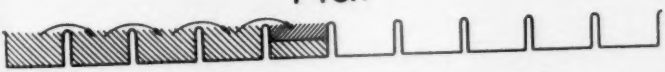

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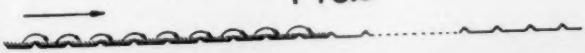

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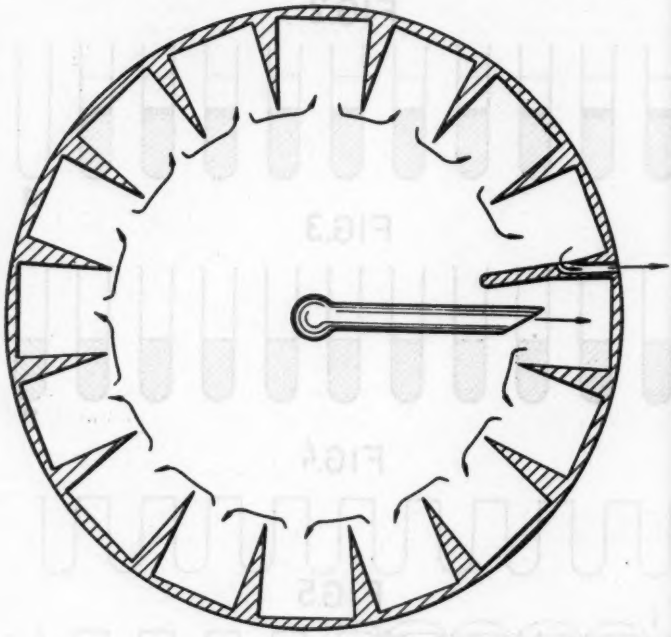
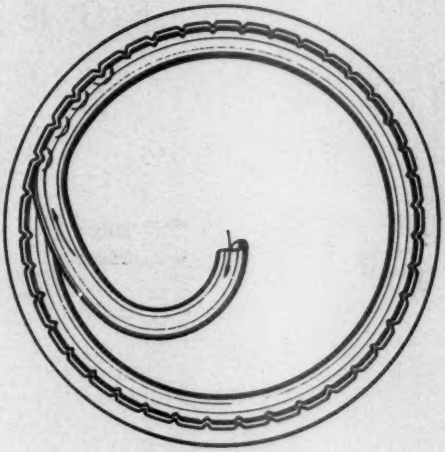


FIG.10



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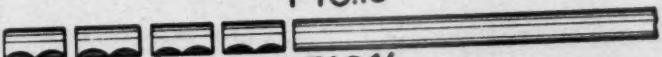

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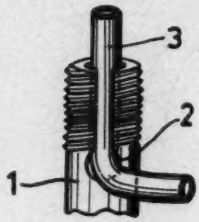
FIG. 15



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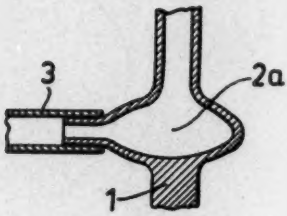

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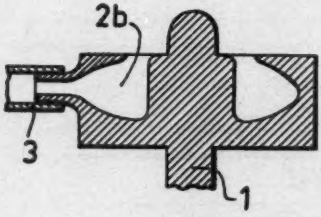


FIG. 18

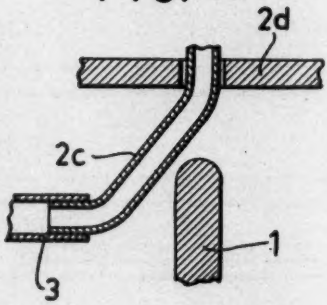


FIG. 19

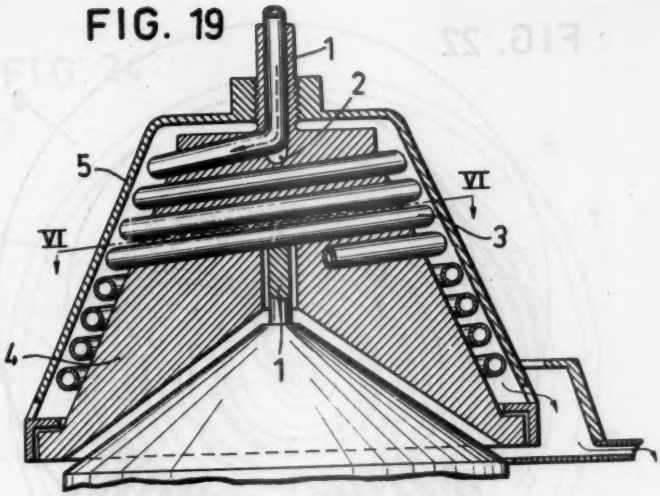


FIG. 20

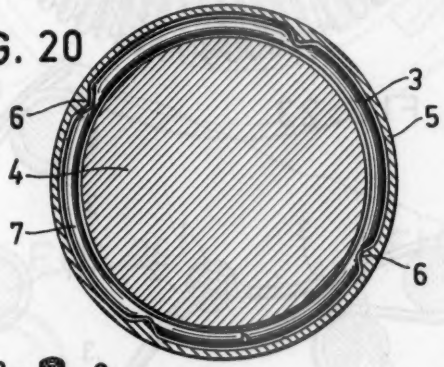


FIG. 21

FIG. 22

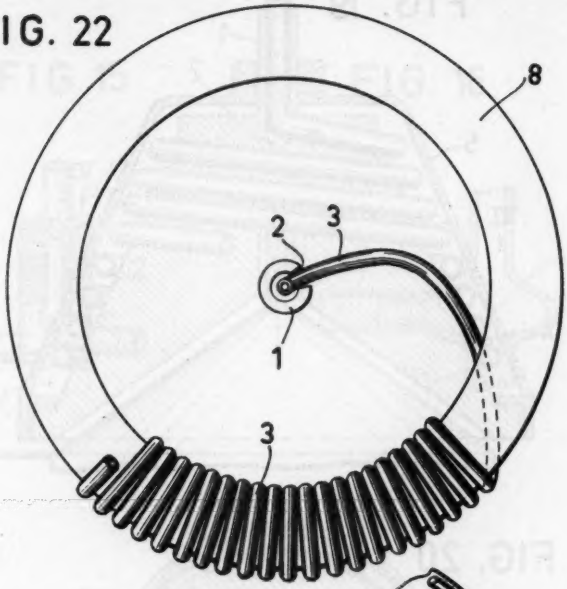


FIG. 23

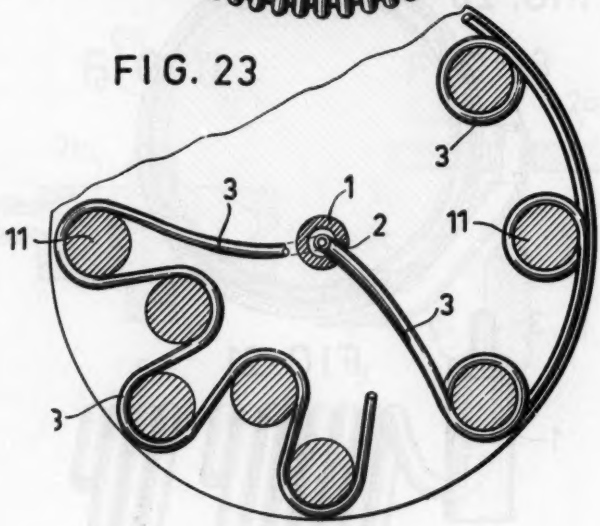


FIG. 24

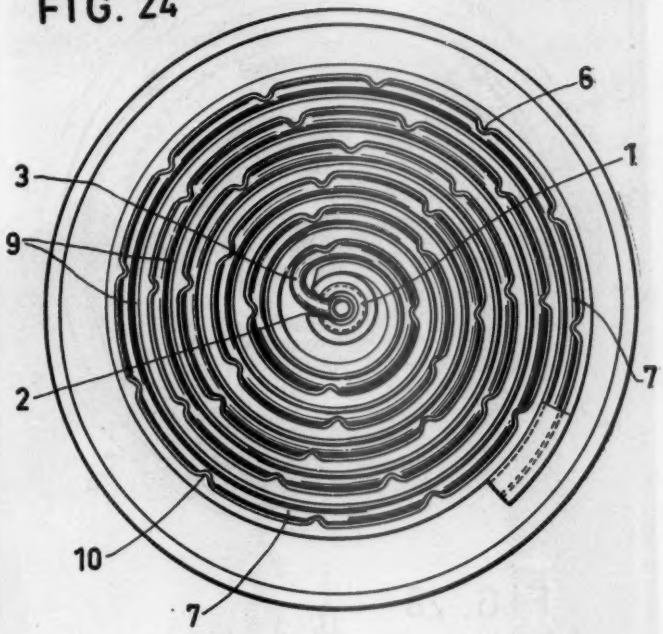


FIG. 26

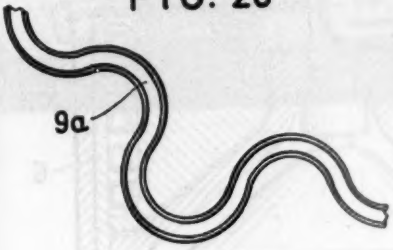


FIG. 25

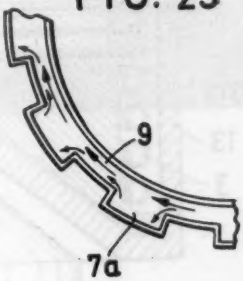


FIG. 27

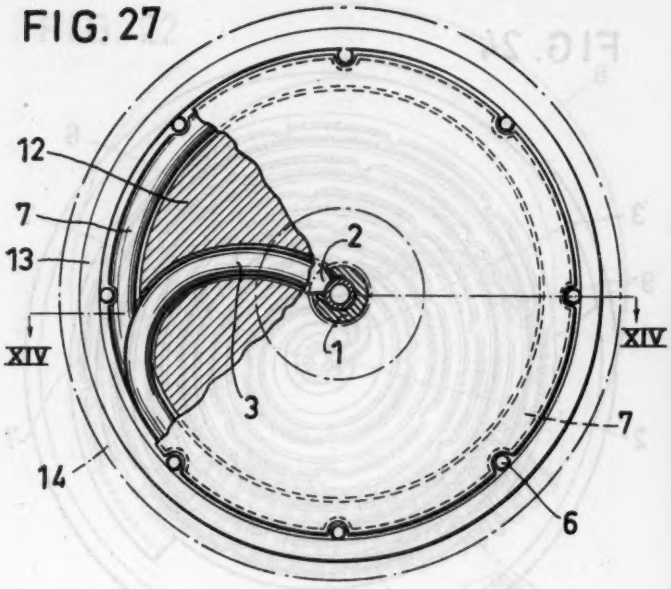


FIG. 28

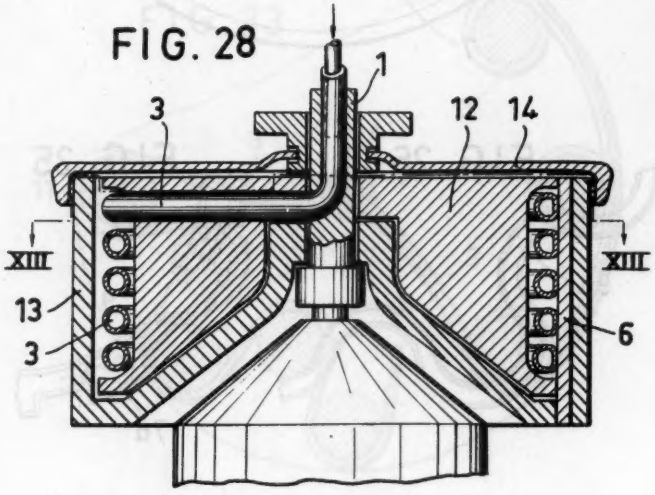


FIG.29

FIG. 30

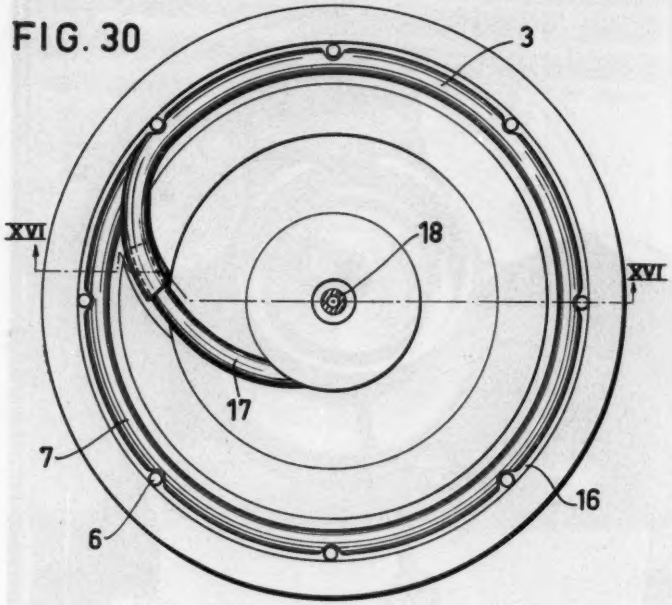


FIG. 31

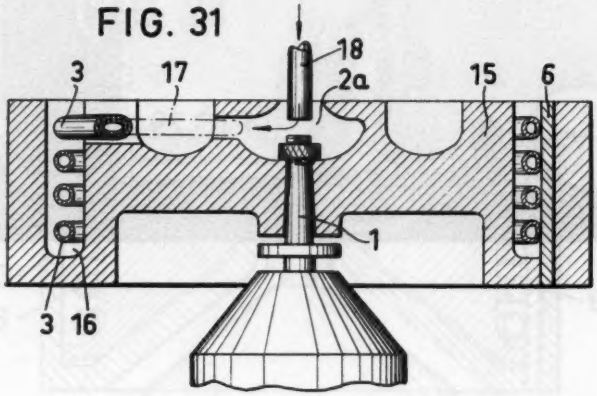


FIG. 32

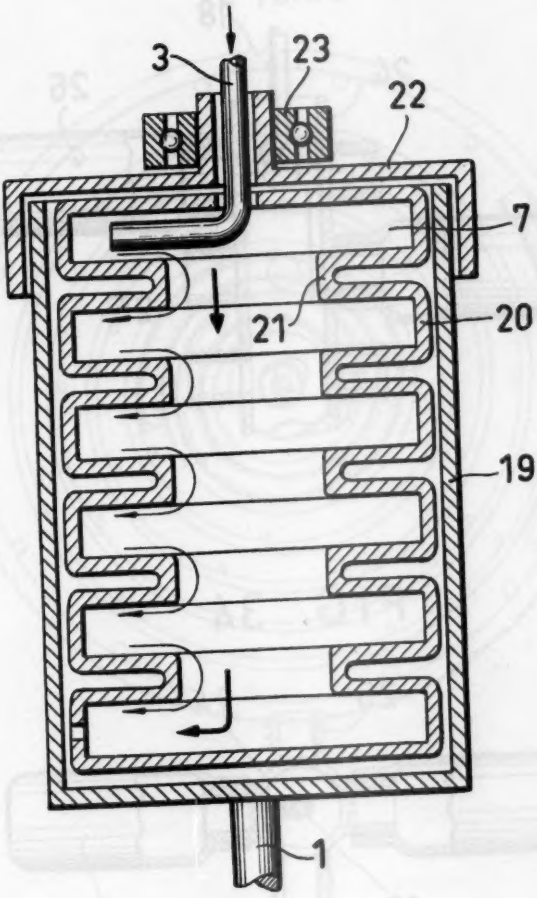


FIG. 33

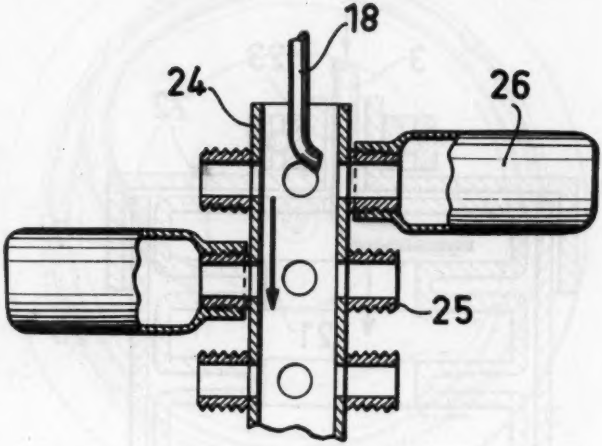


FIG. 34

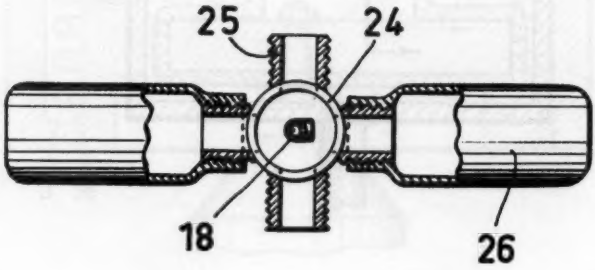


FIG.35

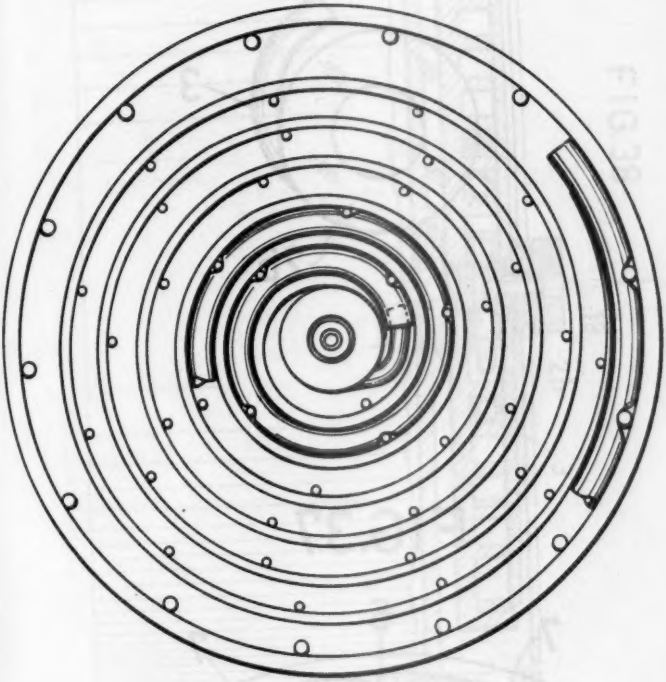


FIG.36

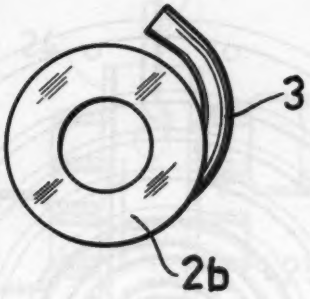


FIG.37

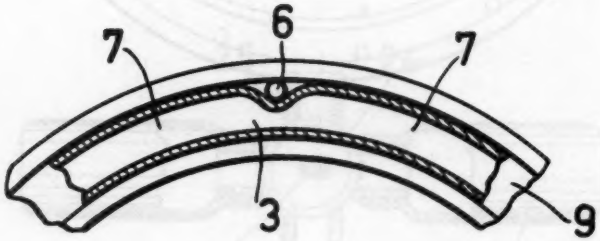


FIG.38

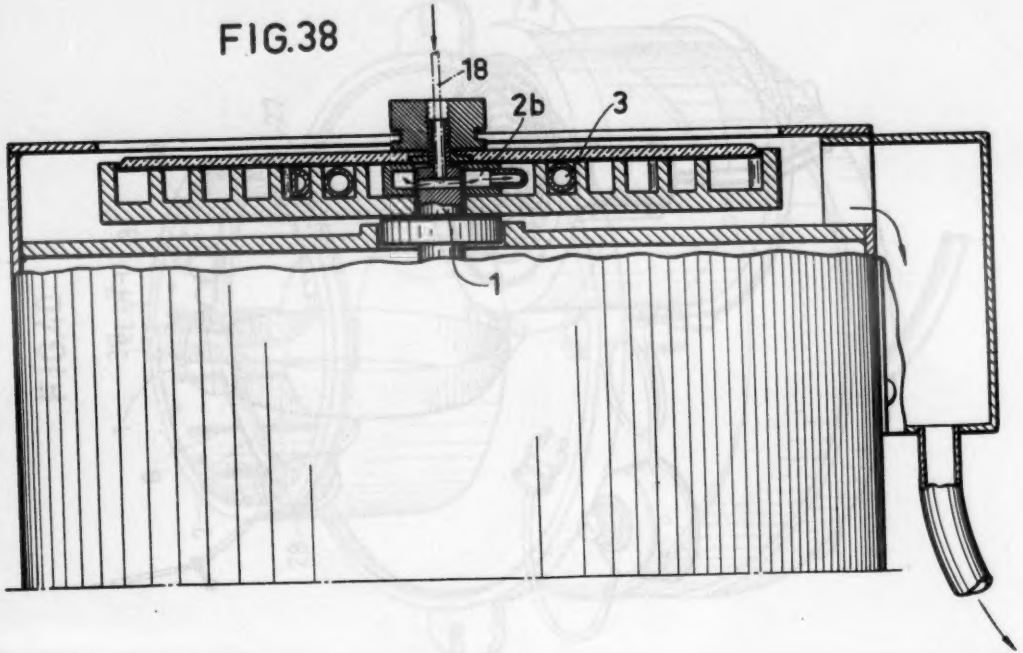


FIG.39

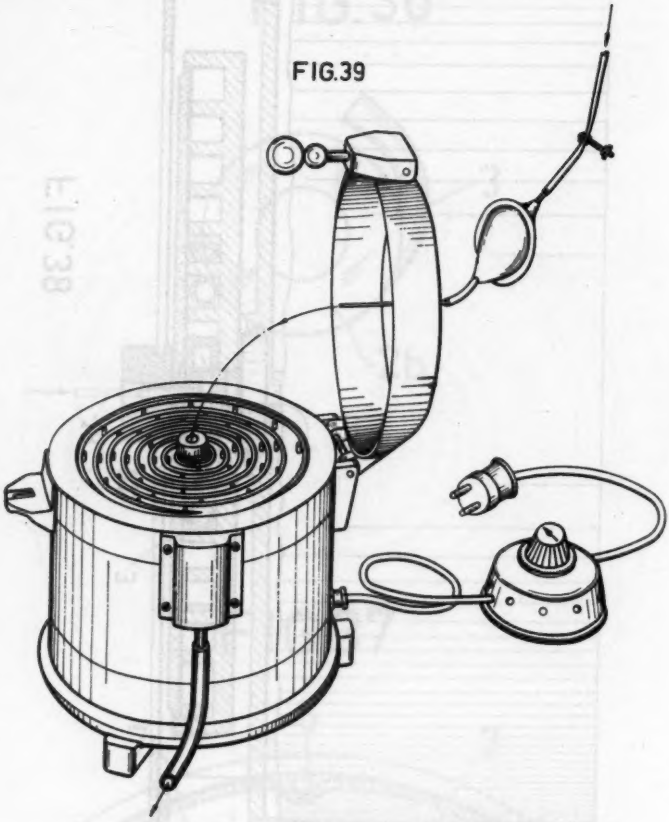


FIG.40

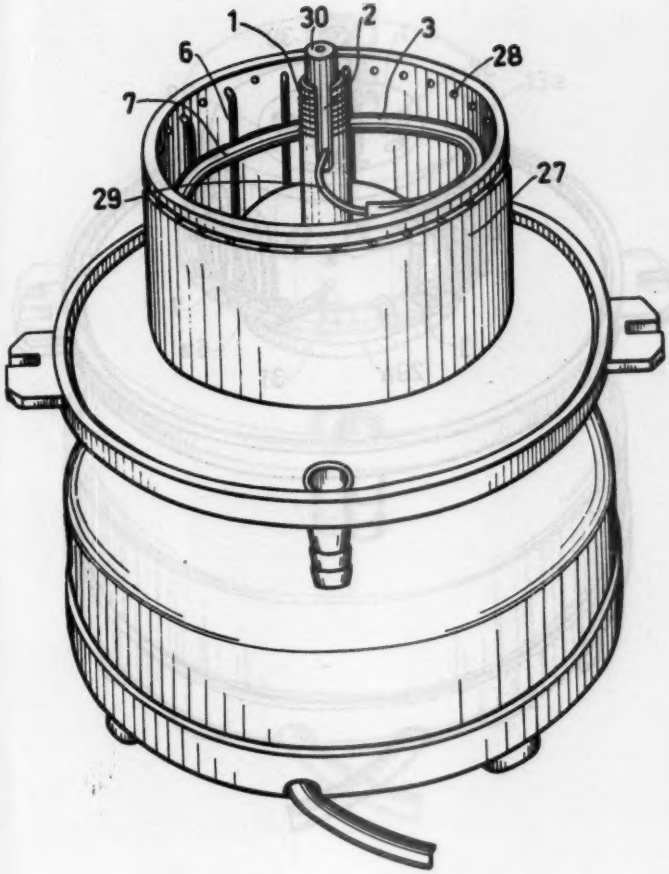


FIG.41

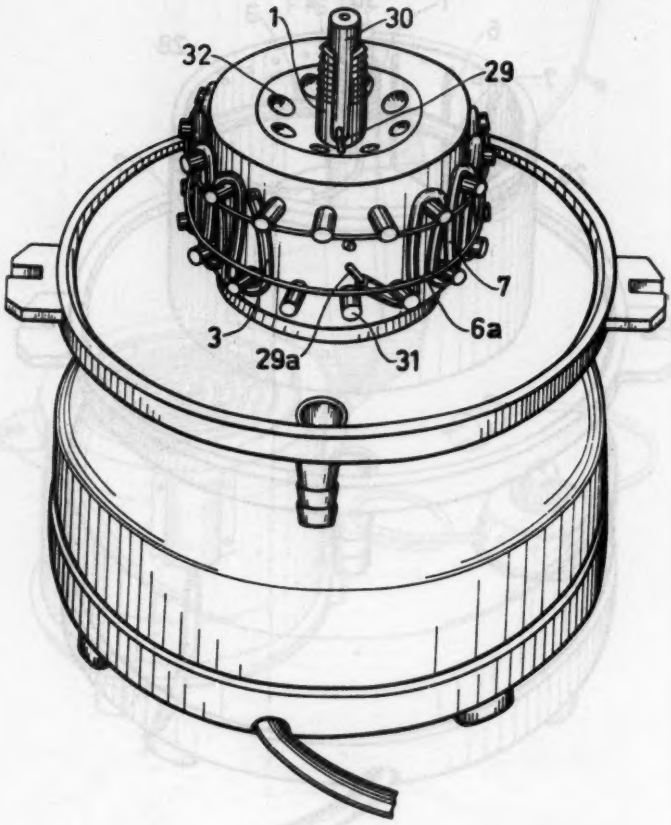


FIG.42

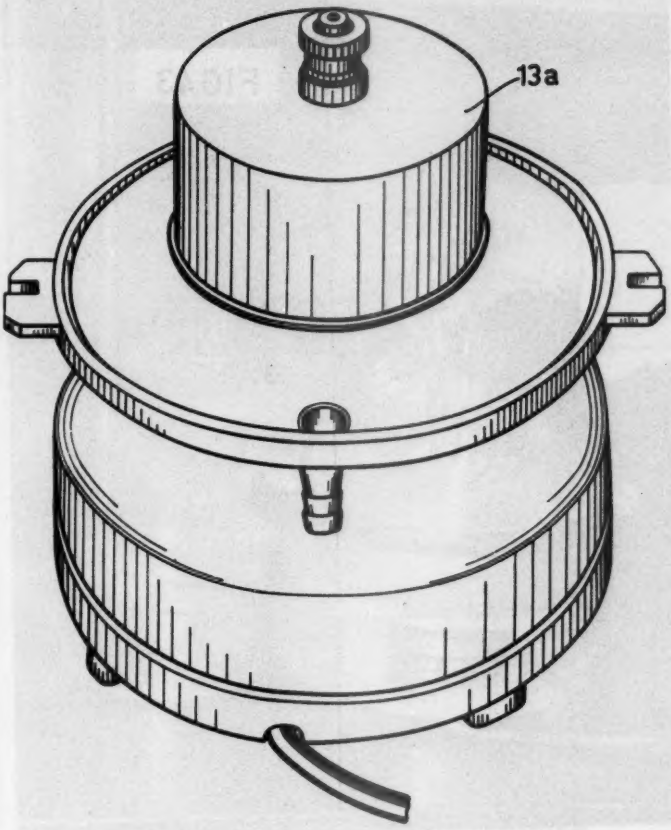
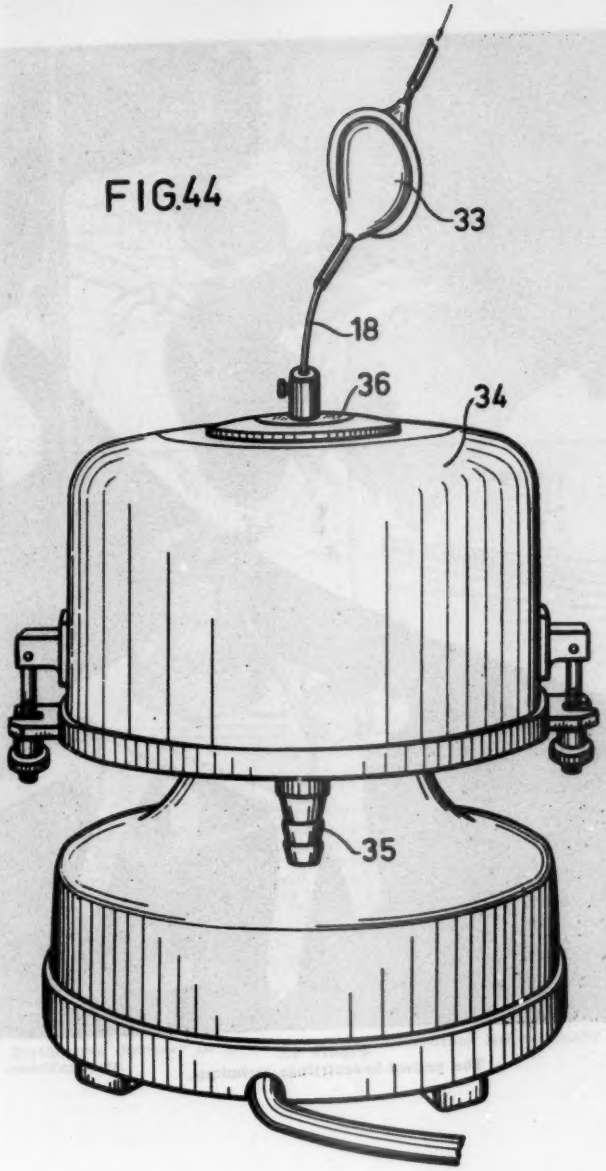


FIG.43

FIG.44



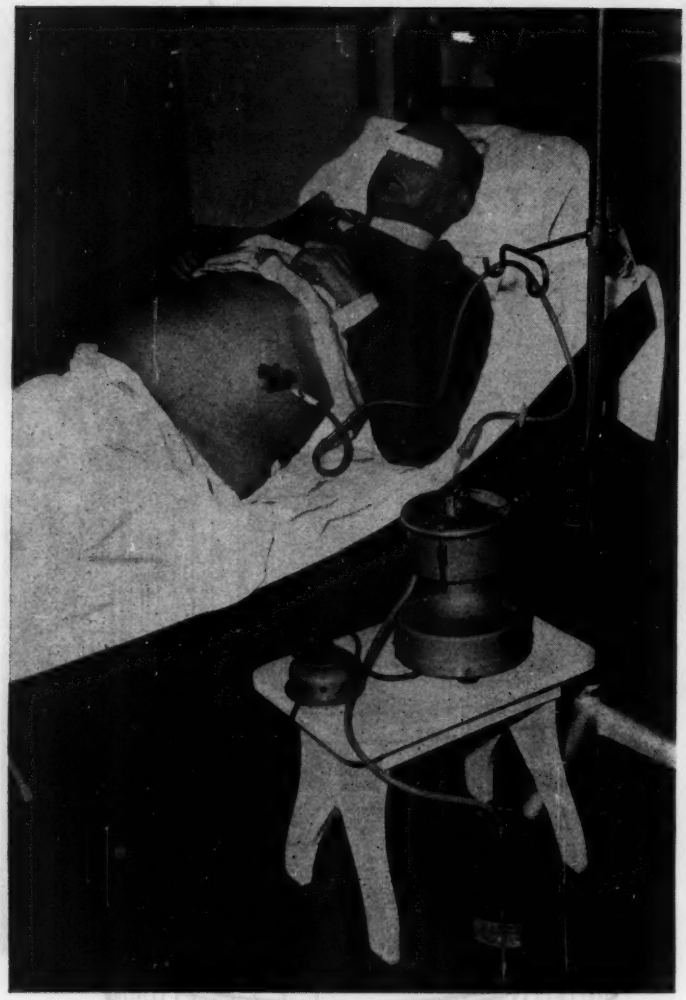


Figure 45.
The patient-to-centrifuge technique.

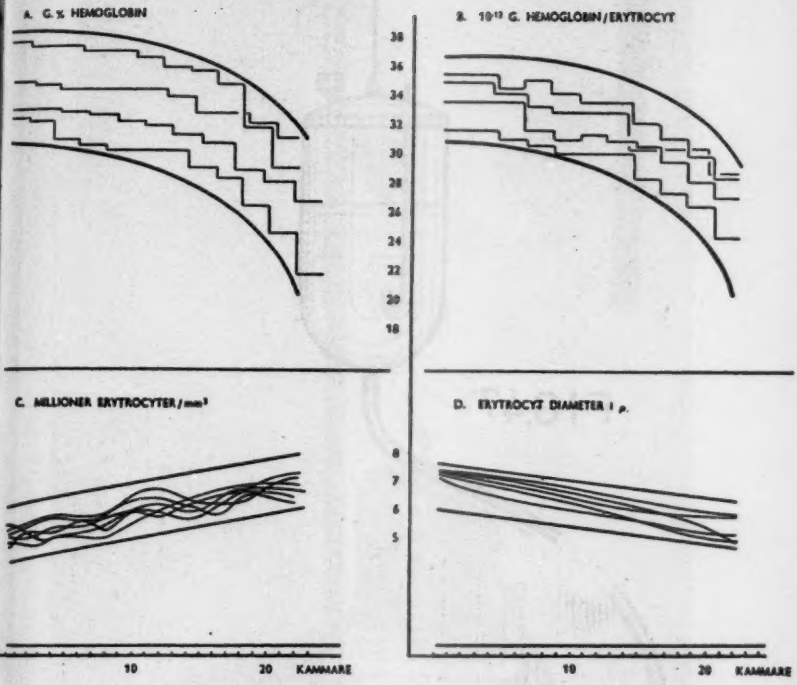


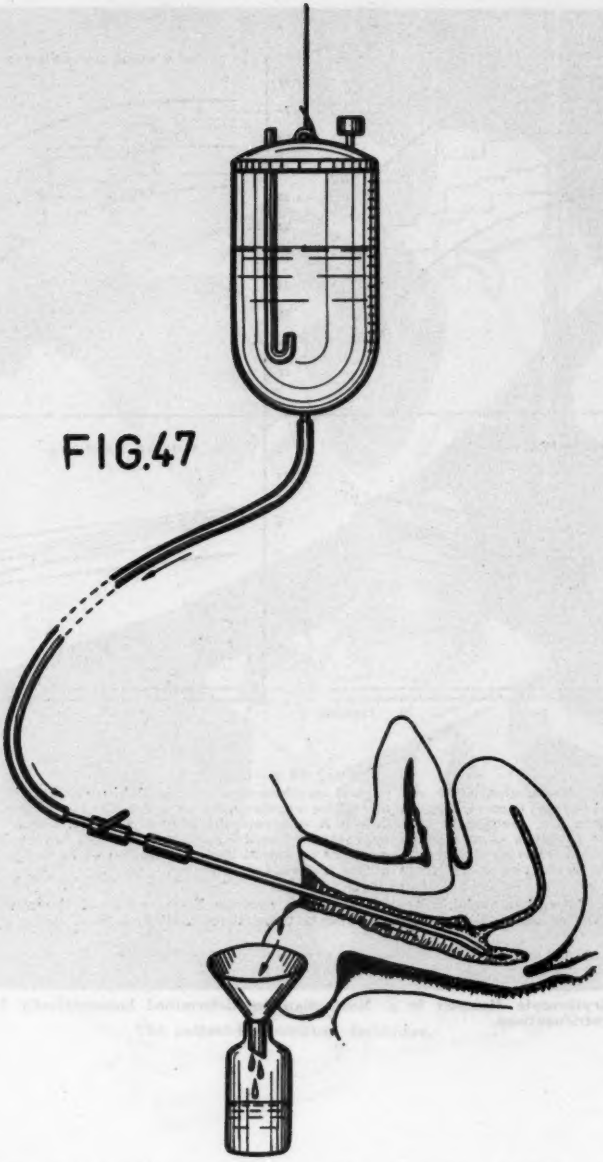
Figure 46 a—d.

a. Gr. Haemoglobin/100 ml packed erythrocytes. Haemoglobin content is determined by haemolysing 10 microlitre erythrocytes in 5 ml Drabkin's cyanide solution and reading in a Beckman B spectrophotometer at 540 mμ. Upper and lower contours of the curve represent 26 centrifugations of erythrocytes from different individuals. Stepwise curves represent the distribution of haemoglobin, in different chambers, from a few individuals.

b. 10^{-12} Gr. haemoglobin/erythrocyte. The contour lines represent the absolute amount of haemoglobin per erythrocyte from 10 centrifugations.

c. Millions of erythrocytes per mm³. The distribution represents the number of erythrocytes per mm³ from 10 centrifugations.

d. Erythrocyte diameter in μ. Mean diameter determined halometrically from 5 centrifugations.



Collection of exfoliated cells by vaginal washings with isotonic (physiological) salt solutions.

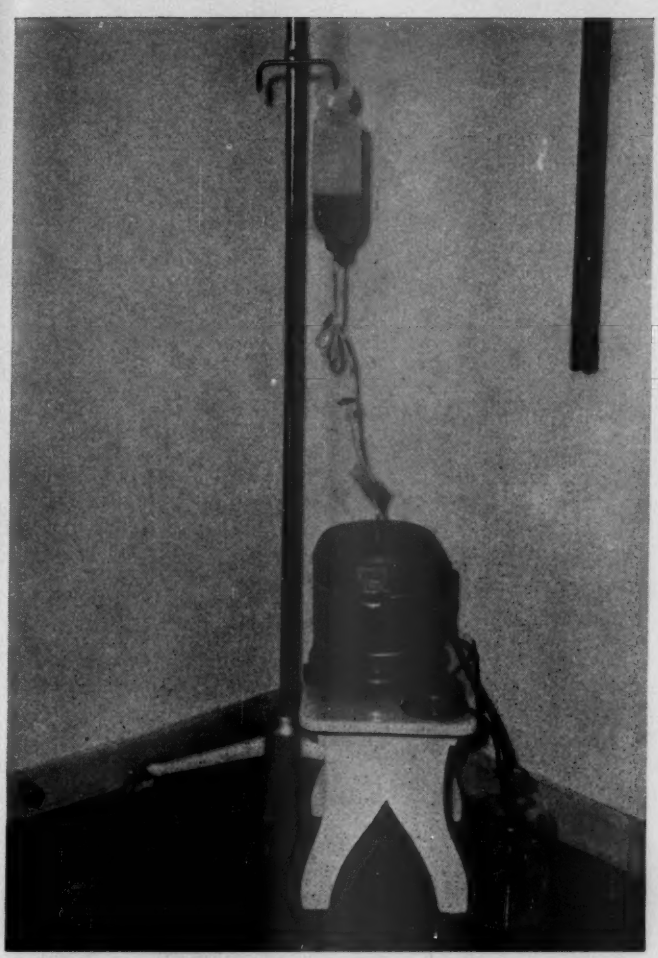


Figure 48.

Arrangement for collection and continuous centrifugation of urine. The patient voids urine into the funnel leading the suspension to the rotating centrifuge. A capacity of 20—30 litres per hour can be attained at 6,000—8,000 r.p.m., and an ordinary urine sample is centrifuged within a few minutes. Urine cleared of solid particles can be collected in a bottle connected to the outlet of the centrifuge. After centrifuging the sample the tubing is removed from the rotor and the proximal end of it is used for cytological, and the distal end, showing a thin semitransparent film on the peripheral side, for bacteriological examination.